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Regulatable promoters of *Saccharomyces cerevisiae*: comparison of transcriptional activity and their use for heterologous expression

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Saccharomyces cerevisiae has become a popular model system for studying the molecular biology of the eucaryotic cell. Many mutants of *S. cerevisiae* have been successfully used for the isolation of functional homologues from other species by heterologous complementation. All these analyses require the ectopic and regulated expression or coexpression of genes or cDNAs at different levels under the control of heterologous promoters. Inducible and regulatable expression of proteins is of great importance especially for the comparative analysis of dosage dependent effects . In this paper, we compare the expression rates from different regulatable promoters at low or high copy number. Based on these data we have designed a series of compact vectors for the convenient cloning of genes and their regulated expression at different levels and in various genetic backgrounds.

For the comparison of the expression rates we chose the promoters derived from the genes encoding O-acetyl homoserine sulphydrylase (MET25; ref. 2) and galactokinase (GAL1; ref. 3). The MET25 promoter is repressed when cells are grown in the presence of methionine (4). The GAL1 promoter is tightly repressed by glucose and is strongly induced by galactose (3). Since the expression from the very strong GAL1 promoter can lead to toxic effects we constructed two deletion variants termed GALL and GALS which lack either one or one and a half of the 3 UAS elements required for full induction of the promoter by galactose (5).

Promoters were cloned as PCR-generated SacI/XbaI fragments into the low copy vector pRS416 (6) or the high copy vector pRS246 (7) which already carried a PCR-generated *XhoI*/*Kpn*I fragment of the CYC1-terminator. The resulting plasmids were termed pRS416prom or pRS426prom (Figure 3). In order to compare the expression rates achieved by the different vectors, a lacZ gene was cloned as a *XbaI*/*Bam*HI cassette downstream of the promoters and the β- galactosidase activity was determined in the strain YPH499(6) as described in (8). In the case of the MET25 promoter lacZ expression was measured in the presence of increasing amounts of methionine. As can be seen from Figure 1, expression varied from 55OU for the 2μ plasmid in the absence of methionine to 9U for the centromeric plasmid in the presence of 1 mM methionine. The promoter responded in a logarithmic way to increasing methionine concentrations. The repression of the MET25 promoter by 1 mM methionine resulted in a rest activity of 10% as estimated by the activity of the very stable β-galactosidase. For proteins with a short half live we found only 1% of residual activity (data not shown).

Expression rates of the different GAL1 promoter derivatives were determined in the repressed state when cells were grown in the presence of glucose or in the induced state when cells were grown in absence of glucose and presence of galactose. Figure

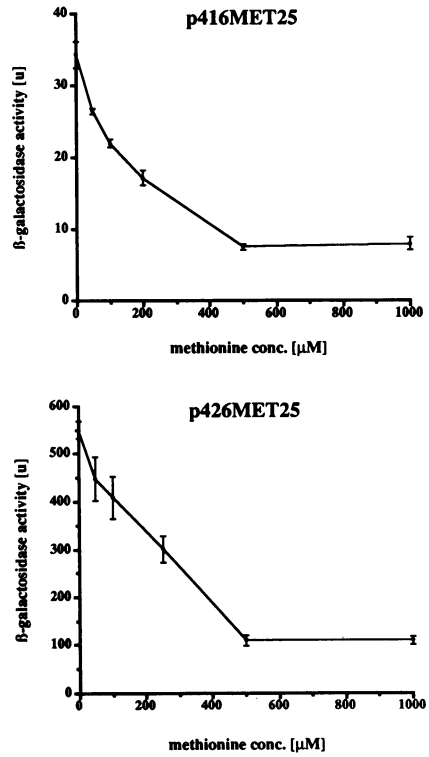

Figure 1. Repression of the MET25 promoter expression. The β-galactosidase activities of the MET25 promoter/lacZ fusions in the plasmids p416 or p426 were determined at different methionine concentrations as described in (8). Values represent the average of 5 independent colonies tested.

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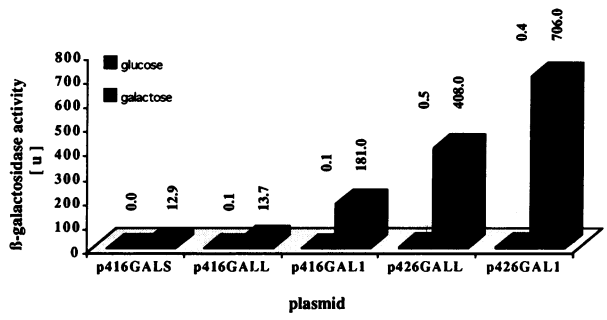
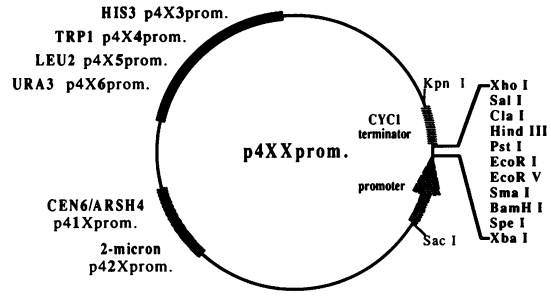


Figure 2. Induction of expression levels from plasmids carrying different GAL1 promoter derivatives. The β- galactosidase activities of the GAL promoter/lacZ fusions in the plasmids p416 or p426 were determined in glucose or galactose medium. Cells were pregrown in a medium containig 2% of raffinose and then shifted to raffinose medium supplemented either with 2% of glucose or 4% of galactose. Values represent the average of 5 independent colonies tested.

A



B

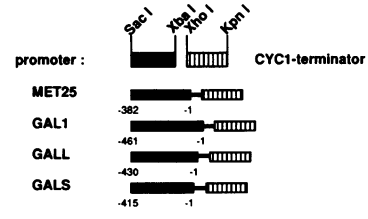


Figure 3. Structure of the expression vectors. Part **A** shows the schematic map of the plasmids constructed. The nomenclature is based on the plasmids described by Christianson *et al.* (7). For example plasmid p424MET25 carries the MET25 promoter and is based on PRS424 carrying the TRP1 gene and the 2 μ origin of replication. Shown are the restriction sites of the polylinker (bold) between the terminator and the promoter (arrow). Part **B** shows the maps of the different promoters (dotted box) and the CYC1 terminator (11, striped box). Numbers at the boxes represent the regions of the promoters cloned by PCR relative to the start codon (+1 would be A of the ATG codon).

2 shows that none of the expression vectors exhibited a significant activity under repressing conditions. Release of repression and induction by galactose led to an expression which varied from 1OU for the centromeric GALS promoter to 80OU for the 2μ GAL1 promoter. The truncated versions of the GAL1 promoter showing a low but fully repressible activity are ideally suitable for the conditional expression of genes that are essential for yeast cells but that would be toxic when overexpressed by the normal GAL1 promoter.

Finally the different expression cassettes (Figure 1B) were cloned into the centromeric and 2μ plasmids (p41Xprom and p42Xprom; Figure 1A) of the pRS series (6,7) carrying a HIS3, TRP1 or LEU2 marker gene (p4X**3**, p4X**4**, or p4X**5** in Figure 1A). The multiple cloning array of these plasmids based on pBIISK (Stratagene, La Jolla, CA) provides 6 to 9 unique cloning sites depending on the plasmid backbone (for further information contact the authors). This polylinker allows the construction of cDNA libraries by the directional cloning of cDNAs generated by the widely used ZAP system (Stratagene, La Jolla, CA).

In summary, we have determined the activity of different regulatable *S. cerevisiae* promoters which are either completely repressible as in the case of the GAL promoter and its derivatives, or can be gradually repressed with some remaining rest activity, as in case of the MET25 promoter. We subsequently constructed total of 32 yeast expression vectors that allow for the regulated expression of heterologous proteins at various levels over a range of 2−3 orders of magnitude. The multiple cloning array provides up to 9 unique restriction sites for the convenient cloning of genes or cDNAs. The different marker genes of the vectors facilitate the expression in different genetic backgrounds. They also provide a powerful tool to study the co-expression of up to four different genes at various levels. Many of the vectors described here have already been used successfully in our laboratory for the expression of various murine and human cDNAs (9) and fusion proteins (10).

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